rapid-quenching techniques to independently study these processes in adenylate kinase (ADK) and to characterize their energetic contribution at atomic resolution. Adenylate kinase is an important enzyme that catalyzes a reversible reaction: 2ADP ↔ ATP +AMP, and is involved in maintaining energy homeostasis in a cell. Two binding sites of ADK and three different ligands result in a variety of biochemical states (determined by the bound ligands). Each of these states has different dynamic properties making the overall structural dynamics of ADK complex. We have tackled this problem by engineering a loss-of-function ADK mutant (with greatly reduced rate of turnover), which allowed us to ''trap'' the enzyme in well-defined biochemical states. Our results lead to a detailed energy profile of ADK, providing insights into the molecular mechanism of its functioning. This work reveals fundamental principles of enzyme catalysis and highlights the role of protein's intrinsic dynamics.

95-Plat

Quadrupolar-Order Deuterium NMR Relaxation Provides New Light on Dynamics of Retinal in Rhodopsin

Andrey V. Struts[1], Gilmar F.J. Salgado[2], Michael F. Brown[1].
[1]Department of Chemistry, University of Arizona, Tucson, AZ, USA, [2]Département de Chimie, École Normale Supérieure, Paris, France.

Deuterium NMR relaxation of quadrupolar order (R_{1Q}) was applied to study dynamics and ligand-protein interactions underlying rhodopsin activation. Rhodopsin was regenerated with retinal ^{2}H-labeled at the C5-, C9-, and C13-methyl groups and recombined with POPC bilayers [1-3]. The ^{2}H NMR relaxation rates were measured in the dark state of rhodopsin in the temperature range from −30 to −150 °C. In our previous studies, we measured relaxation rates of Zeeman order (R_{1Z}) in the dark, Meta I, and Meta II states of rhodopsin. The dynamical parameters involve the spectral densities of motion, which depend on correlation times and can be expressed in terms of a pre-exponential factor (rotational diffusion constant, D_0, or jump rate, k_0, for mobility in absence of a barrier) and the corresponding barrier height (activation energy E_a). The values of D_0, k_0, and $E_a$describe local packing of retinal within the binding pocket of rhodopsin and the changes occurring in the activation process. The R_{1Z} relaxation rates enable determination of dynamical parameters, but they do not establish the anisotropy of methyl rotation. Simultaneous fitting of temperature dependences of the R_{1Z} and R_{1Q} relaxation rates indicates the off-axial rotation of the methyl groups is at least an order of magnitude slower than the axial motion. The new R_{1Q} data confirm previous conclusions on methyl group dynamics and their interactions with the binding pocket [1]. Taken together, the R_{1Z} and R_{1Q} data allow the motional spectral densities to be individually determined, and afford a new way of investigating the dynamics of ligand-protein interactions in membranes. [1] A.V. Struts *et al.* (2010) *Nature Struct. Mol. Biol.* (in press). [2] G.F.J. Salgado *et al.* (2006) *JACS* **128**, 11067. [3] A.V. Struts *et al.* (2007) *J. Mol. Biol.* **372**, 50.

96-Plat

Allostery and Folding Mechanisms of the N-Terminal Receiver Domain of Protein NTRC

Swarnendu Tripathi[1,2], John J. Portman[2].
[1]Rensselaer Polytechnic Institute, TROY, NY, USA, [2]Kent State University, Kent, OH, USA.

The nitrogen regulatory protein C (NtrC) of enteric bacteria plays a central role in the control of genes involved in nitrogen metabolism. Nuclear Magnetic Resonance (NMR) studies suggest that the N-terminal receiver domain of NtrC ($NtrC^r$) exhibits slow conformational dynamics in the microsecond timescale. Allostery in this protein occurs by shifting the preexisting population from the inactive to active state upon phosphorylation (see Fig. 1.). Using a coarse-grained variational model, we give a site specific description of both the folding and conformational transition mechanisms of this βα-repeat protein. Differences in the folding mechanisms to the active and inactive state are consistent with a significant stabilization of the helix-α4 upon activation. Our model suggests that the allosteric conformational change of $NtrC^r$ involves a marked decrease in the flexibility of this protein upon activation without local partial unfolding. In particular, we find that in addition to the functionally important helix-α4, the β3α3 loop also plays significant role in the inactive/active conformational transition mechanism of $NtrC^r$.

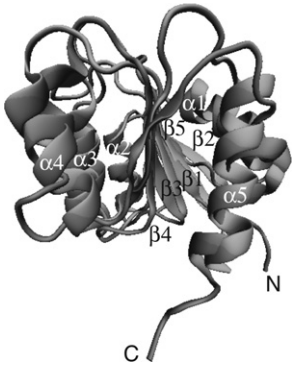

Fig. 1. Phosphorylation induces large conformational change in the NtrCr. The unphosphorylated (inactive) structure is shown in blue and the phosphorylated (active) structure in red.

97-Plat

Novel Protein Semi-Synthesis Methods for Monitoring Conformational Dynamics

E. James Petersson.
University of Pennsylvania, Philadelphia, PA, USA.

One of the great challenges facing biochemists is to understand the rapid and complex structural dynamics of proteins. Fluorescence measurements can be made on the ns timescale, and distance-dependent interactions such as Förster resonant energy transfer (FRET) can be used to determine the separation of chromophore labels to glean time-resolved structural information on protein motions. However, the relatively large size of common fluorophores precludes assigning these motions at atomic resolution. We have recently demonstrated that a thioamide, a single-atom substitution of the peptide backbone, can be used as a fluorescence quenching probe to monitor structural changes in proteins. We are using these small chromophores to examine the folding of model proteins and working to extend these methods to full-size proteins through semi-synthesis methods.

98-Plat

Ric-8a Catalyzed G Protein Activation Proceeds Through a Disordered State

Stephen R. Sprang[1], Celestine Thomas[1], Klara Bricknarova[1], John Sumida[2], Gregory Tall[3].
[1]University of Montana, Missoula, MT, USA, [2]University of Washington, Seattle, MT, USA, [3]University of Rochester, Rochester, NY, USA.

Members of the Ras superfamily of regulatory GTP binding proteins, Heterotrimeric G protein alpha subunits (Ga) undergo cycles of activation and deactivation driven by binding and hydrolysis of GTP. Activation occurs by replacement of GDP by GTP at the nucleotide binding site of Ga, which requires catalytic assistance from guanine nucleotide exchange factors (GEFs). Transmembrane G protein-coupled Receptors (GPCRs) are the best known G protein GEFs, but recently, a novel family of cytosolic, non-receptor GEFs, typified by mammalian Ric-8A, were discovered. Unlike GPCRs, which act on G protein heterotrimers, Ric-8A catalyzes the release of GDP directly upon Gi-class Ga subunits (Gai), and has negligible affinity for Gai-GTP. Upon binding to Gai-GDP, Ric-8A catalyzes GDP release and forms a stable Gai:Ric-8A complex that dissociates only in the presence of GTP, resulting in the release of Gai-GTP. The TROSY-HSQC spectrum of [1H,15N]Gai bound to Ric-8A is considerably broadened relative to Gai-GDP. Hydrogen-deuterium exchange mass spectroscopy shows that the rate of HD exchange at Gai:Ric-8A is more than 2X faster than from Gai-GDP. Differential scanning calorimetry shows that both Ric-8A and Gai-GDP undergo cooperative, irreversible unfolding transitions at 47 deg and 52 deg, respectively, while nucleotide-free Gai shows a broad, weak transition near 35 deg. The unfolding transition for Gai:Ric-8A is complex, with a broad transition peaking at 49_i. Ric-8A therefore stabilizes nucleotide-free Gai in a dynamic state, which, we propose, facilitates GTP binding. We show that the C-terminus of Gai is a critical binding element for Ric-8A, as is known to be the case for GPCRs, suggesting that these two GEFs act by similar mechanisms as chaperones for the unstable and dynamic nucleotide-free state of Ga.

99-Plat

Protein Dynamics at the Picosecond-Nanosecond Time Scale: a Complementary Study by Dielectric Spectroscopy, Neutron Spectroscopy and MD Simulation

Sheila Khodadadi[1], Joseph E. Curtis[1], Alexei P. Sokolov[2,3].
[1]NIST, Gaithersburg, MD, USA, [2]Oak Ridge National Laborat, Oak Ridge, TN, USA, [3]University of Tennessee, Knoxville, TN, USA.

We have studied dynamics of hydrated protein ($h \sim 0.4$ grams of water per gram of protein) in picoseconds-nanosecond time scale using dielectric spectroscopy, neutron spectroscopy and molecular dynamics (MD) simulations. We have observed two relaxation processes in dielectric spectra of hydrated protein: ''main'' (tens of picoseconds) and ''slow'' (nanoseconds). Traditionally these processes have been attributed to the relaxation of bound hydration water and not to the protein. Using Neutron scattering data, the ''main'' process has been assigned to the protein-water coupled motion. MD simulations focused on protein relaxation processes in picoseconds-nanosecond time range also revealed protein motions at the same time-scale as the processes observed in dielectric spectra of hydrated protein. Detailed analysis of the MD simulations and comparison to dielectric data indicate that the observed relaxation process in the nanosecond time range is mainly due to the protein. The relaxation processes involve the entire structure of the protein, including atoms in the protein backbone, side chains and turns. Both surface and buried protein atoms contribute to this motion, however surface atoms demonstrate slightly faster dynamics. Analysis of the water atom residence times reveals that 90% of hydration water exchange with the bulk on time scale shorter than 100 ps, and indicates that there are not enough stationary water molecules at the protein surface to support the bound water-only interpretation of the observed dielectric process in nanosecond time range.